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EVALUATION OF FLUORIDE UPTAKE IN THE ENAMEL OF PRIMARY TEETH AFTER USING DIFFERENT CONCENTRATIONS OF A GREEN TEA EXTRACT: AN *IN-VITRO* STUDY

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ABSTRACT: It is believed that extracts of green tea increase fluoride uptake in deciduous enamel and the aim of this *in vitro* study was to evaluate the fluoride uptake in the enamel of primary teeth after using different concentrations of an extract of green tea. Seventy-five enamel samples were prepared from deciduous canine teeth of children in Kerman, Iran, and randomly divided into five groups of 15: Group 1 (G1): no treatment (negative control); Groups 2 (G2), 3 (G3), 4 (G4), and 5 (G5) were treated with green tea extract in concentrations of 0.2%, 1%, 2%, and 10%, respectively. After the surface treatment, all the samples were submitted to an acid challenge consisting of daily immersion for 5 days in a demineralizing (3 hr) and a mineralizing solution (21 hr). The fluoride concentrations were then calculated with a potentiometer. One-way ANOVA was used for statistical analyses ($p<0.05$). There was a significant difference between the fluoride uptake in the control and all other groups except for the G2 group (0.2% green tea extract) ($p<0.05$). There was no statistically significant difference between the fluoride uptake in the enamel in the G2 (0.2% green tea extract and the G3 (1% green tea extract) groups. The findings reveal that green tea can increase the amount of fluoride in the enamel of the primary teeth.

Keywords: Deciduous teeth; Enamel; Fluoride uptake; Green Tea.

INTRODUCTION

Tea is one of the favorite drinks all over the world, especially in Iran, and is produced from the *Camellia sinensis* plant species. It contains polyphenols such as tannin, caffeine, flavonols, theine, and aromatic components.[1] In addition, it has been shown that green tea can prevent cardiovascular diseases, obesity, and cancer and has antioxidative, anti-aging, antimicrobial, and anticariogenic potentials.[2,3] No specific side-effects have been reported due to the use of green tea.[2] Significant amounts of fluoride are found in the tea plant (almost 200–1000 mg/kg), and 98% of the fluoride is concentrated in the leaves.[4.5] Fluoride easily passes into tea infusions and is transferred to the tooth enamel during drinking tea.[4.5] Dry tea leaves contain 4–400 ppm of fluoride and brewed tea contains 0.34–6 ppm fluoride.[6] Ramsey et al. reported an inverse relationship between the tea use rate and the DMFT index in children, which was attributed to the fluoride found in tea.[7] Simpson et al. reported that after cleaning the oral cavity with tea, approximately 66% of the fluoride remained in the saliva and the dental plaque, with the capacity to bond to the oral cavity hard tissues.[6]

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Currently, the anticariogenic activity of local fluoride has been confirmed. Up to a point, it does not result in fluorosis and it has been the subject of several comprehensive reviews and meta-analyses. The main mechanism of action of fluoride is through an increase in the remineralization process of the decalcified mineral structures of teeth, followed by an increase in the resistance of tooth structures against the subsequent acid attacks.[8] Studies of the chemical reactions of concentrated fluoride solutions with the enamel show that the reactions occur on the external surface of the enamel, which results in increased resistance of this surface to demineralization.[9,10] One of the useful effects of fluoride in the oral cavity is its local effect on the enamel of newly erupted teeth. Therefore, the daily exposure of tooth enamel to low concentrations of fluoride results in anticariogenic effects.[11,12] Poureslami et al. showed that the amount of fluoride in the enamel of primary teeth in children living in areas with high concentrations of fluoride in water and foodstuffs is up to 108.7 ppm.[13] Although the prevalence and severity of dental caries have decreased significantly in recent years, millions of children and adults still lose their teeth due to dental caries and experience malocclusion. In Iran, despite widespread activity to prevent and control dental caries, studies have shown a high rate of dental caries.[1] Possibly the most important mechanism through which green tea prevents dental caries is the provision of large amounts of fluoride for the enamel, increasing its resistance against caries. However, it is possible that tannin, too, might have a role in preventing dental caries through its antibacterial properties. Fluoride, in addition being in water and food, can also enter the body through the air.[14] An excessive amount of fluoride in the body can have harmful effects. These harmful effects have been discussed in numerous articles.[15-19]

The present study was undertaken to evaluate the absorption of fluoride from different concentrations of green tea extract by the enamel of primary teeth. It is hoped that the results of the preset study will be used to apply a safer fluoride therapy in children. To the best of our knowledge, no similar study is available in scientific databases.

MATERIALS AND METHODS

In the present *in vitro* study, 75 sound primary canine teeth, extracted for orthodontic treatment were selected from the teeth of children referred to the Faculty of Dentistry, Kerman University of Medial Sciences, Kerman, Iran. The teeth were stored in 1% normal saline solution (NSS) until used for the purpose of the study. The inclusion criteria consisted of no use of local fluoride therapy during the previous 1-year period, no history of the use of fluoride supplements, and no use of fluoride supplements by the children's mothers during pregnancy. Before the extraction of the teeth, the parents signed informed consent forms for the use of their children's teeth in the research project. A square, measuring 3×3 mm, was drawn on the crown of each tooth. The squares were separated from the crowns using a disk in a hand piece. The depth of each square was 1 mm and only consisted of enamel. Before initiating the procedures, the enamel surface was evaluated under a magnifying glass (CAMAR, Iran) for the presence of any cracks and teeth with cracks were excluded.

The green tea extract was prepared by a chemist in the Faculty of Pharmaceutics, Kerman University of Medical Sciences. To this end, fresh green tea leaves produced in Iran in 2017 (in Lahijan in the north of Iran) were procured

and after their physical and herbal properties were confirmed by a biopharmacologist, they were used to prepare green tea extract at different concentrations. The dried green leaves were powdered in a mortar. A hundred grams of the powder was mixed with 500 mL of water, which was passed through a filter after 48 hours to eliminate the precipitate. The remaining solution was kept at room temperature; after 4 days, the green tea extract powder was achieved. To prepare the 0.2% aqueous extract of green tea, 0.04 g of the powder was dissolved in 20 mL of distilled water. In addition, for the 1% and 2% concentrations, 0.2 and 0.4 g of the powder were dissolved in 20 mL of distilled water. To achieve a 10% concentration, 10 g of green tea powder were dissolved in 100 mL of distilled water.[20] The samples were randomly assigned to 5 groups (n=15). The samples size in each group was 15 based on similar studies and based on the advice of the statistician.[20]

Group 1: No surface treatment was carried out and the group served as a control.

Group 2: In this group, the 0.2% green tea extract was used. The tooth surface was cleaned with a prophylaxis brush, dried with an air syringe, and then the extract was applied on the prepared tooth surface with the use of a soft brush. Then the tooth surface was dried again and the extract remained in contact with the tooth surface for 4 minutes. The contact of any moisture with the tooth surface was prevented.

Group 3: The surface preparation procedures in this group were similar to those in group 2; then 1% green tea extract was used on the tooth surfaces.

Group 4: The surface preparation procedures in this group were similar to those in group 2; then 2% green tea extract was applied to the tooth surfaces.

Group 5: The surface preparation procedures in this group were similar to those in group 2; then 10% green tea extract was applied to the tooth surfaces.

After completion of the surface treatments, all the samples in the 5 study groups underwent a pH cycling procedure. The samples were immersed in a demineralizing solution (consisting of 1.0 Mm of lactic acid and 6% hydroxyethyl cellulose with a pH of 4.5) for 3 hours.

Then the samples were immersed in a mineralizing solution (consisting of 0.03 g/L of $MgCl_2$, $6H_2O$, 0.121 g/L of K_2HPO_4, 0.625 g/L of potassium, 0.05 ppm of fluoride, 2.0 g/L of methyl-p-hydroxybenzoate, 0.4 g/L of sodium carboxymethyl cellulose, with a pH of 6.7) for 21 hours. These cycles were repeated for 5 consecutive days and the enamel samples were stored in a solution containing deionized water until they were analyzed. The demineralizing and mineralizing solutions contained 99% methyl hydroxybenzoate to prevent the growth of fungal species. Subsequent to these procedures, the potentiometric technique with the use of a specific fluoride ion electrode was used to determine the amount of fluoride in each block in the Faculty of Pharmaceutics, Kerman University of Medical Sciences. To this end, the samples underwent a biopsy procedure using the acid etch technique. Seventy-five plastic containers with lids were prepared for sampling, which contained 1 mL of 0.5-mol perchloric acid ($HClO_4$). One tooth sample was immersed in each container with the use of pliers. The biopsy from each window was carried out for 30 seconds and the tips of the pliers were covered with nail varnish resistant to acid to prevent ionic interactions with the tips, which could disrupt the solutions'

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integrity. The samples were shaken gently during the biopsy procedures to prevent the return of the released fluoride ions to the enamel.

Then the enamel was rinsed with 2 mL of 0.2-mol potassium hydroxide (KOH). The enamel surface was completely dried with a small cotton pellet. Then the cotton pellet was transferred into the test container that contained the solution resulting from the biopsy procedure so that the dissolved enamel would be completely collected. To eliminate any ionic interference or any possible interferences, 1 mL of the biopsy sample of each sample was retrieved to determine the amount of fluoride and diluted 10 times. In addition, in order to eliminate the interference of the OH ion during the determination of the amount of fluoride using the potentiometric technique, the pH was adjusted in the mild acidic range (around 6–6.5) and TISAB buffer was added to the solutions during the dilution technique. After preparing the potentiometer, the electric potential of each concentration was read using a fluoride ion specific electrode.[20] The test was performed twice for each sample and the mean of the two tests was recorded as the amount of fluoride in the sample after drawing the standard curve.

RESULTS

The amount of fluoride in 0.2%, 1%, 2%, and 10% green tea was 0.6 ppm, 0.9 ppm, 1.7 ppm, and 5.5 ppm, respectively. Table 1 presents the fluoride content in the 4 groups with different concentrations of green tea and in the control group.

Table 1. Fluoride content (µg/mL) in the four green tea groups and in the control group

Sample number	Group				
	Control group	Green tea group			
		0.2 %	1%	2%	10%
1	0.218	0.467	0.421	0.857	1.011
2	0.117	0.323	0.379	0.996	1.235
3	0.316	0.359	0.485	0.547	1.398
4	0.267	0.562	0.428	0.621	1.490
5	0.147	0.428	0.363	0.769	0.682
6	0.89	0.275	0.258	0.732	0.952
7	0.406	0.184	0.729	0.951	1.262
8	0.053	0.347	0.681	0.861	0.789
9	0.133	0.226	0.718	0.791	1.371
10	0.169	0.113	0.365	0.371	1.213
11	0.228	0.589	0.602	1.090	1.461
12	0.176	0.101	0.264	0.939	0.498
13	0.096	0.382	0.360	0.501	1.087
14	0.235	0.472	0.597	0.829	0.957
15	0.194	0.264	0.108	0.628	0.781
Average	0.189604	0.33947	0.45053	0.76553	1.07913

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One-way ANOVA was used to reveal significant differences between the groups and showed significant differences between the different study groups ($p<0.05$). The results showed significant differences between the control group and all the other groups except for group 2 (0.2% green tea). Table 2 presents comparisons between the study groups in the uptake of fluoride by enamel.

Table 2. Comparison of the fluoride uptake between the green tea (GT) groups and the control group at $p<0.05$ (NS=not significant)

Group	Group				
	Control group	Green tea group			
	G1 (0% GT)	G2 (0.2% GT)	G3 (1% GT)	G4 (2% GT)	G5 (10% GT)
G1 (0% GT)	–	NS	p<0.05	p<0.05	p<0.05
G2 (0.2% GT)	NS	–	NS	p<0.05	p<0.05
G3 (1% GT)	p<0.05	NS	–	p<0.05	p<0.05
G4 (2% GT)	p<0.05	p<0.05	p<0.05	–	p<0.05
G5 (10% GT)	p<0.05	p<0.05	p<0.05	p<0.05	–

DISCUSSION

Considering the infectious nature of dental caries, which is one of the most prevalent orodental diseases, it is necessary to undertake studies in order to decrease the prevalence and severity of this condition in children and adolescents. Green tea, apart from its rinsing properties, contains large amounts of fluoride and it has been shown that fluoride absorbed by the tooth enamel, at a level that does not result in fluorosis, increases the resistance of enamel to the acid attack by cariogenic bacteria.

The results of statistical analyses showed significant differences in the fluoride uptake by enamel between the different study groups. The maximum fluoride uptake was detected with the use of 10% green tea extract, followed by the 2%, 1%, and 0.2% concentrations and the control group, respectively. Despite the difference in the mean fluoride uptake between the 0.2% concentration group and the control group, the difference between these two groups was not significant. The mean fluoride uptake in group 3 (1% green tea extract) was higher than that in group 2 (0.2% green tea extract); however, the difference was not significant. It appears the lack of a significant difference between groups 2 and 3 might be attributed to the fact that the mere release of fluoride by the green tea extract and the presence of the fluoride ion in the vicinity of the enamel (even at low concentrations) is enough for the fluoride uptake by the enamel. Therefore, although these two groups exhibited a difference in the amount of released fluoride, the difference in fluoride uptake by the enamel was not significant between these two groups. In addition, it should be pointed out that an increase in samples size might result in a significant difference between the two groups.

The present study showed that the *in vitro* use of 2% and 10% concentrations of green tea extract resulted in the release of adequate amounts of fluoride ion, followed by its uptake by the enamel. This indicates that penetration of fluoride into the enamel structure occurs subsequent to the use of these two concentrations, with a higher uptake with the use of 10% green tea extract, which was significant based on the results of statistical analyses. Since the mean fluoride uptake in group 2 (0.2% green tea extract) was higher than that in the control group, with no significant difference between these two groups, it might be concluded that the amount of fluoride released from the 0.2% green tea extract was not sufficiently high to be absorbed by the enamel. Although is no similar study available for proper comparison, we compared the results of the present study with the available studies on tea consumption and its relationship to the dental health.

Schmidt et al. evaluated the fluoride content of deciduous teeth after the regular use of black tea and reported that the regular use of black tea containing fluoride resulted in a definite increase in the fluoride content of these teeth. These researchers considered tea as an effective material in the prevention of dental caries.[21] Although this study by Schmidt et al. evaluated black tea, the results are consistent with those of the present study with green tea. In a study in Kouhbanan, Kerman, Iran, the mean fluoride content of deciduous teeth in children with grade 3 or 4 fluorosis (based on the Dean index) was determined to be 108.7 µg/mL (ppm), which was attributed to the high fluoride content of the drinking water, foodstuffs and vegetables in that area.[13] In the present study, the mean fluoride content of the enamel in the deciduous teeth of children in Kerman in group 5 (10% green tea extract) was 1.07 µg/mL (ppm). Since the fluoride content of drinking water in Kerman is less than the optimal level (0.1–0.3 ppm),[13] it can be concluded that the uptake of fluoride by the deciduous teeth was due to the fluoride present in green tea.

Suyama et al. evaluated remineralization of enamel lesions and inhibition of erosions resulting from acid attack after use of fluoride-containing chewing gum produced from green tea. The results showed that such a chewing gum resulted in higher levels of remineralization and resistance to acid attack compared to a placebo chewing gum and they concluded that the regular use of fluoride-containing chewing gum helps prevent dental caries. Some researchers have attributed the anticariogenic properties of green tea to a phenolic compound, i.e., catechin, in its structure. However, in the study by Suyama et al. the maximum and minimum concentrations of catechin were 0.12 and 0.006 g/dL, respectively. Therefore, they concluded that such concentrations cannot prevent dental caries. They attributed the improvements in the remineralization process and inhibition of erosion due to acids to the release of fluoride from the chewing gum.[22] The results of the present study are consistent with those of Suyama et al. in relation to the uptake of fluoride from tea by the tooth enamel.

Daneshyar et al. evaluated the effect of green tea varnish on the depth of root surface caries. They suggested that use of green tea varnish every 24 or 48 hours for 21 days can prevent caries. The mechanisms of fluoride uptake on the root surface and an increase in resistance to caries were mentioned as the reasons for such an effect,[23] consistent with the results of the present study.

Rudhosh et al.[24] reported that green tea had the best effect on the remineralization of teeth and, in descending order, black tea, *Mentha spicata* tea, and tea with *Psyllium acium* had the best effect on the remineralization of enamel incipient caries,. Bozorgi et al. compared green tea and black tea in relation to microhardness and the prevention of demineralization of the enamel in deciduous teeth. The results showed that use of green tea can increase microhardness and green tea and black tea can increase the resistance of the enamel of deciduous teeth to demineralization.[25] The results of the our study are consistent with the results of both of these studies.

CONCLUSIONS

In general, the results of the present study showed that 2% and 10% green tea extracts had definite effects on the uptake of fluoride by enamel, indicating that green tea results in an increase in the amount of fluoride in the enamel of deciduous teeth. In addition, the fluoride in green tea can have an important role in decreasing the prevalence and severity of dental caries if it is used as an oral rinse at a proper concentration.

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